Does Neuronal Activity Promote Glioma Progression?

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Does neuronal activity promote glioma progression?

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Abstract

Excessive glutamate release by glioma cells induces pharmacologically accessible neuronal hyperexcitation, including epilepsy. Two recent reports suggest that neuronal hyperexcitation stimulates *bona fide* glutamatergic synapses on glioma cells. Ionotropic glutamate receptors activate intercellular calcium signaling networks to orchestrate growth and invasion, presumably by facilitating oncogenic signaling cascades and cytoskeletal remodeling.

Main text

In the adult brain, neuronal activity induces neuroglial stem and progenitor cell proliferation and directs their migration via glutamatergic synapses. Two recent publications indicate that a similar mechanism may be hijacked by glioblastomas [1] and diffuse midline gliomas [2], two devastating malignant brain tumor entities with a generally fatal clinical course. Combined ultrastructural and electrophysiological studies identified *bona fide* glutamatergic synapses between neuronal axon terminals and post-synaptic glioma cells. In xenograft models, signaling through such neuron-glioma synapses promoted tumor growth and invasiveness whereas interference with synaptic transmission had cytostatic effects [1, 2].

Activation of post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type ionotropic glutamate receptors on glioma cells led to membrane depolarization and calcium influx. In normal brain cells the regulatory subunit GluA2 blocks calcium conductance of AMPA receptors. Adenosine-to-inosine editing of the Q/R site of the GluA2-encoding pre-mRNA is required to encode this calcium-blocking property, but underediting in some glioma cells rendered AMPA receptors calcium permeable [1, 2].

Non-synaptic spontaneous inward currents have also been identified in glioma cells. These smaller, but longer lasting potassium currents through inward rectifying channels are activated in response to potassium leakage from firing neurons and can trigger calcium

transients via voltage-gated conductances, albeit in a less efficient way than synaptic transmission [1, 2].

In normal neuronal tissues, N-methyl-D-aspartate (NMDA) type glutamate receptors are the major source of post-synaptic calcium currents. Continuous activation of NMDA receptors due to extensive glutamate secretion by glioblastoma cells can induce calcium-mediated neuronal apoptosis. By contrast, glioma cells are resistant to high extracellular glutamate concentrations because they lack significant levels of NMDA receptor expression [1, 2].

In gliomas, inward calcium transients propagate through intercellular networks via gap junctions [1, 2]. These networks are formed by cytoplasmic extensions termed tumor microtubes and have been proposed to convey growth signals and resistance to conventional chemoradiotherapy [3]. Most synapses between axonal endings and glioblastoma cells form along these tumor microtubes [1].

Of note, oligodendrogliomas, a less aggressive form of diffusely infiltrating glioma, do not form tumor microtube networks [3]. Nonetheless, single cell gene expression analyses identified expression of post-synaptic genes comparable to astrocytomas, suggesting alternative mechanisms of electric tumor cell activation [1]. Future studies will determine if and how neuronal activity exerts similar tumor propagating effects in non-astrocytic gliomas.

Only approximately half of the cells in glioblastoma and diffuse midline glioma express post-synaptic gene signatures, and neuron-glioma synapses are present on an even smaller proportion of cells [1, 2]. Single cell gene expression analyses of human diffuse midline gliomas identified a hierarchical cellular structure with a subpopulation of glioma cells characterized by enrichment of an oligodendrocyte precursor cell (OPC) signature [4]. Glioma cells exhibiting enrichment of this OPC signature also exhibited enriched expression of post-synaptic genes. These findings indicate that synapses between axons and glioma cells formed preferentially on undifferentiated cell populations, whereas the bulk of more differentiated tumor cells was widely devoid of post-synaptic gene expression [2]. Whether a

functional link between synaptic transmission and the stem-like phenotype exists remains to be determined.

The identification of synaptic and non-synaptic electric interaction between neurons and glioma cells closes a loop of multifaceted pro-tumorigenic functions of glutamate in glioma biology (Figure 1). Extensive glutamate secretion by glioma cells via the cystine/glutamate exchanger system x_c drives hyperexcitability of neurons in the tumor microenvironment [5], along with autocrine and paracrine stimulation of tumor cell growth and invasion via AMPA type glutamate receptors [6]. Enhanced neuronal activity promotes shedding of neuroligin-3 into the synaptic cleft [7], which activates oncogenic signaling cascades and induces the expression of genes involved in synaptic transmission in glioma cells in a paracrine manner [8]. Subsequent synapse formation links neuronal hyperexcitability and epilepsy to tumor progression.

Whether epilepsy is indeed an unfavorable predictor in glioma patients remains controversial. Exploring associations of epilepsy and patient survival is challenging and requires precise annotation of confounders that can skew results. For example, brain tumors presenting with epilepsy are more likely to be diagnosed at an early stage of the disease course when usually no symptoms other than epilepsy are present. Tumors associated with epilepsy may also be surgically better accessible, because proximity to the cerebral cortex is a pre-requisite for the induction of seizures, and many epileptogenic brain areas such as the temporal or frontal cortex can be resected relatively safely. Epilepsy is also more frequent in patients with prognostically more favorable isocitrate dehydrogenase mutated gliomas. Vice versa, sub-clinical neuronal hyperexcitation may contribute to tumor progression independently of whether or not epileptic seizures occur. Anti-epileptic therapy with valproic acid or levetiracetam was not associated with outcome in a pooled secondary analysis of 1’896 glioblastoma patients who were treated in four different randomized clinical trials [9], but this study did not account for the presence of epilepsy or other confounders mentioned further above.

Nonetheless, several clinically approved drugs could be repurposed to target the neuronal activity-driven tumor-propagating mechanism of gliomas (Figure 1). Lowering extracellular glutamate levels with the anti-epileptic drug and inhibitor of branched chain amino acid transaminase 1 (BCAT1) gabapentin [10], or with the anti-inflammatory drug sulfasalazine [5], an inhibitor of the cystine/glutamate antiporter system x_c may reduce autocrine AMPA receptor activation, neuronal hyperexcitability and NMDA-mediated neuronal cell death. Neuroprotective NMDA inhibition can also be attempted with the anti-dementia drug memantine. The anti-epileptic drug perampanel, an inhibitor of AMPA-type glutamate receptors, and genetic modulation of AMPA type receptors exerted anti-invasive effects in xenograft models [1, 2]. Disruption of calcium networks by pharmacological inhibition of gap junctions utilizing the clinically approved anti-inflammatory drug meclofenamate likewise inhibited growth and migration mediated by synaptic transmission [2]. Lastly, neuroligin-3 shedding can be targeted utilizing pharmacologic inhibitors of the sheddase ADAM10 (A Disintegrin And Metalloprotease Domain 10), thus preventing the activation of synaptic gene expression programs and direct activation of growth factor signaling cascades in glioma cells [8].

In summary, a glutamate-driven vicious circle links neuronal hyperexcitation and epilepsy to tumor growth and conveys several drugable vulnerabilities of brain tumors for which very little progress has been made in the last two decades.

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Figure legend

Figure 1. Pharmacologic targets at the neuron-glioma interface. Physiologically, neuronal action potentials depolarize synaptic end-feet, which activates voltage-gated calcium channels (not shown) to drive neurotransmitter release into the synaptic cleft. The excitatory neurotransmitter glutamate binds post-synaptic AMPA and kainate type ionotropic

receptors, which conduct sodium inward and potassium outward currents, resulting in membrane depolarization. The GluA2 subunit of AMPA type receptors prevents calcium influx in neurons, and upon combined repetitive chemical stimulation and membrane depolarization, calcium currents through slow-kinetics NMDA type receptors are activated to promote synapse formation between neurons (not shown). Excessive BCAT1-dependent glutamate secretion by glioma cells through the glutamate/cystine antiporter system Xc promotes neuronal hyperexcitability, which can result in epilepsy and may induce calcium-mediated neuronal apoptosis via overactivation of NMDA-type glutamate receptors. Glioma cells lack NMDA receptors, but express calcium-conducting AMPA receptors that are stimulated by glutamate in an autocrine and paracrine fashion to promote tumor growth and invasion, presumably through activation of oncogenic signaling cascades and cytoskeletal remodeling. Neuronal activity also promotes shedding of neuroligin-3, which activates oncogenic signaling cascades including the focal adhesion kinase (FAK) and phosphoinositide 3-kinase (PI3K) pathways, and drives synaptic gene expression in glioma cells in a paracrine manner by acting on one or several unknown receptors. Glioma cells are interconnected in a tubular network by gap junctions. Glutamatergic synapses form on tumor microtubes and activate calcium-conducting AMPA receptors. Calcium signals of tumor cells may thus be propagated to connected cells, further driving growth and invasion. Several pharmacologic inhibitors of different nodes of this neuron-glioma interface are clinically approved for other indications (red writing).

Physiologic synapse

Neuroligin-3 shedding

Post-synaptic gene expression program

Hyperexcitability epilepsy

Ca-induced apoptosis

Synaptic AMPA-R activation

Ca2+ signal propagation

Autocrine AMPA-R activation

Cystine

Glutamate

Glutamate

Na+

K+

Ca2+

Neuroligin-3

ADAM10

?

FAK

PI3K

Synaptic genes

Growth invasion

Under clinical developement

Perampanel

Ca2+

Ca2+

Meclofenamate

Ca2+

Growth invasion

Gabapentin

Sulfasalazine

Ca2+

aKG

?

BCAT1

Glutamate

Glutamate

Growth invasion

Cystine

?

Ca2+

Perampanel

Ca2+

Ca2+

Ca2+

Ca2+

K+

K+

Na+

Na+

Anti-epileptic drugs

Memantine

Key:

Tumor cell

Neuron

Xc antiporter

AMPA-R, Kainate-R

NMDA-R

Ca-conducting AMPA-R

Action potential

Glutamate

Tumor microtubes

NLGN3

Gap junction